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A life in research on lens regeneration and transdifferentiation

An interview with Goro Eguchi

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Even at the age of 70, Goro Eguchi maintains a busy schedule: he is the Chairman and President of Shokei Educational Institution in Kumamoto, and flies every Monday morning from Kyushu to Tokyo to attend meetings to decide Japan's science and technology policy as Principal Fellow and Research Mentor of the Japan Science and Technology Agency. In this interview, I wanted to know how he became interested in developmental biology, and what he wanted to discover and achieve as a developmental biologist. I knew he is an excellent illustrator, as he draws all the original figures published in his papers. During our interview, he showed me two beautiful and well-preserved manuscripts which he had copied himself from insect books when he was sixteen (Fig. 1A). This talent in drawing changed his life when the famous developmental biologist Tadao Sato saw Eguchi's illustrations in his histology and comparative anatomy classes. In our interview, he recounted how he learned from Professor Sato and went on to become a developmental biologist, studying lens regeneration in the newt. At 70, he still does experiments on newt lens regeneration, rearing newts from fertilized eggs at his home. As mentioned in this interview, he has recently found that repeated lens removal causes a significant delay in lens regeneration from newt epithelium. He finds time to relax by making model sailboats with his very nimble artist's fingers (Fig. 1C).

When did you first become interested in living organisms?

I was born in 1933 in Nagoya as the fifth son of my father, who succeeded and managed a factory founded by my grandfather. My father had been engaged in marine transportation as the chief navigator of an ocean liner until he took over his father's factory, which was producing wooden parts for many different kind of machines including textile machinery. So he was very intellectual, good at English and had an international outlook. Both of my parents were very fond of natural history, and they bred many species of animals in their garden and ponds. They gave us a good chance to study natural history from books such as Fabre's '"Life of the insect". In such a home environment I gradually became interested in natural history, and I eventually began to collect insects, especially Lepidoptera, when I was ten.

When did you decide to study biology?

While I was at high school, I thought seriously about my future and studied to enter the Nagoya University School of Medicine and to become a medical doctor. In my second year, I took lecture courses in histology and comparative anatomy given by Professor Sato, in the Department of Biology (Figs. 2,3). This was my first communication with him.

Tell me more about Professor Sato.

Professor Sato went to Freiburg in Germany, immediately after graduating from the Department of Zoology at the University of Tokyo, to study embryology under the great Hans Spemann, of the "Spemann-Mangold organizer" (see Sander and Faessler, 2001). He took the problem of lens regeneration in the newt as his research subject, supervised by both Spemann and Otto Mangold, and studied this for 11 years in Germany, publishing some very important papers which are still considered classics of lens regeneration research.

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Fig. 1. Dr. Eguch, scientist and craftsman. (A) *An example of a beautiful manuscript copied by Dr. Eguchi from an insect book when he was only sixteen.* **(B)** *An illustration made by Goro Eguchi in 1954 at the age of 21, which his professor, Prof. Sato asked him to draw for his publication about the life of Hans Spemann.* **(C)** *A more informal and relaxed side of Goro Eguchi at home (2001). One of his favorite hobbies is to make sailboats with his nimble artist´s fingers.*

He was a devout Christian, and warm-hearted. His lectures, if not always exciting, were highly accurate and profound. He gave his students indispensable words, which were essential for any student wanting to become a scientist, and gradually charmed me. One day, he called me into his office. He had very much appreciated my anatomical sketches, and wanted me to draw illustrations for his publication about Hans Spemann's life. I eagerly accepted his proposal, and produced several illustrations (Fig. 1B). Through such communications with Professor Sato, I eventually make up my mind to become a biologist instead of a medical doctor.

What brought you to the research field of lens regeneration?

Professor Sato had never insisted that I study lens regeneration, but gave me absolute freedom to choose my research subjects. When I was in the fourth year class at the Department of Biology, Nagoya University, Professor Sato gave me a paper published by G. Wolff in 1894, entitled "Benerkungen zum Darwinismus mit einem experimentellen Beitragzur physiologie der Entwicklung." In this paper Wolff removed a lens from the newt in order to criticize Darwin's theory of evolution through experimentation, but not to study lens regeneration itself. I was so impressed by and interested in this paper that I eventually decided to take the problem of lens regeneration as my research subject for graduation.

Can you tell me more about that?

I started with the subject given by Professor Sato, lens regeneration in cyclopean eyes in the newt. He had published two papers concerning determination of the fetal choroid fissure and the site of lens regeneration in the iris epithelium. Based on his experimental results, he concluded that the site of lens regeneration was determined at the marginal iris epithelium, just opposite the fetal choroid fissure. However, his conclusion was strongly criticized by Politzer, who claimed that the site of lens regeneration is not always related to determination of the fetal choroid fissure, based on his finding that in a naturally developed cyclops larva the lens was regenerating from the lateral margin of the iris epithelium, corresponding to the dorsal in a normal embryo. Thus, Professor Sato proposed that I should analyze the mode of lens regeneration in cyclopean eyes as my research subject. That was 1956, nearly half a century ago.

So, what did you do?

This was a very difficult field for me, an inexperienced undergraduate. But, I tackled it by systematically analyzing cyclopean eye development, and preparing enough cyclopean larvae to

Fig. 2. Prof. Tadao Sato *shortly before his retirement in 1966 at the age of 63. He lectured in histology and comparative anatomy in the Department of Biology in Nagoya University.*

conduct experiments for lens regeneration using techniques such as microsurgery, vital staining and so on. I carefully traced the developmental process in cyclopean embryos, which could be produced by removing the precaudal plate at the early neural plate stage using the method established by Mangold in 1933. I operated on more than 5,000 newt neurulas, and found that removal of the precaudal plate led to arrest of lateral extension of the presumptive eye anlage, located under the medio-anterior end of the neural plate as a single region, resulting in development of a single optic vesicle at the medio-ventral part of the head region.

I was able to prepare a number of larvae with cyclopean eyes of different degrees of malformation, from cyclopia perfecta and imperfecta to synophthalmia (Fig. 4). In lens regeneration experiments using these larvae, I obtained the following results. First, in cyclopia perfecta and imperfecta which lack the optic stalk, the lens can regenerate from any part of the marginal iris epithelium. Second, in cyclopia perfecta and imperfecta which have the optic nerve, the lens is regenerated mostly from the site of marginal iris epithelium away from the point from where the optic nerve projects out. Third, in synophthalmia, two lenses are always regenerated, one from each lateral iris epithelium.

Those are very interesting results, showing the precaudal plate plays a role in lens development. Were they expected?

These results did not support the conclusions of my teacher and of Politzer, who argued that the site of lens regeneration is predetermined at the dorsal marginal iris corresponding to the lateral marginal iris.

Then I wrote the draft of a paper and showed it to my teacher, Professor Sato. He read it, and got angry and said to me: *"You seriously criticized Politzer's conclusion, but not mine. Why not? This is completely unfair, because my conclusion is also incorrect. I understand your hesitation, because you are my student, but, scientists must be faithful to experimental results. My conclusion should be revised according to your results."* And he proposed: *"If you accept my proposal to write our paper in German, as collaborative work by both of us, I will be very happy to introduce you internationally to scientists in our research field."*

What role did Professor Sato play for you?

I had been deeply impressed by his qualities as a scientist and a university teacher, and so I immediately accepted his proposal. Thus, my first paper was published as only my one written in German and with him as co-author.

From this experience I learned what kind of attitude a young scientist needs and how to write scientific papers. My ability in German was also greatly improved. After that first paper, Professor Sato never put his name on my papers; I did the experiments and wrote the papers by myself, and he recognized that he made no contribution to my publications.

What were your next experiments?

For almost the next ten years, I was engaged in morphological analyses of lens regeneration, using techniques of experimental embryology, electron microscopy, histochemistry, organ culture, and so on.

First, I challenged the hypothesis on lens regeneration proposed by Spemann in 1905. Most of the papers published by 1956, when I started working on lens regeneration in the newt, had supported Spemann's hypothesis. He had presumed that in the newt, first, the neural retina is secreting lens-inducing factors; second, the lens always secretes antagonists to the retinal factors; and third, the iris epithelium originating in the head ectoderm maintains competency to the retinal factors so that lens regeneration can be induced when the antagonists are lost following lens removal.

I performed the following experiments, which exposed essential contradictions in Spemann's hypothesis. When the lens of a larval newt was ventrally shifted and fixed with a thin glass bar, to make a gap between the dorsal marginal iris and the lens surface, a supernumerary lens was formed from the dorsal iris, while the original lens continued to grow. In addition, simultaneous removal of the lens and

Fig. 3. Prof. Tadao Sato *inspiring 4th year students in the Department of Biology at Nagoya University in 1955.*

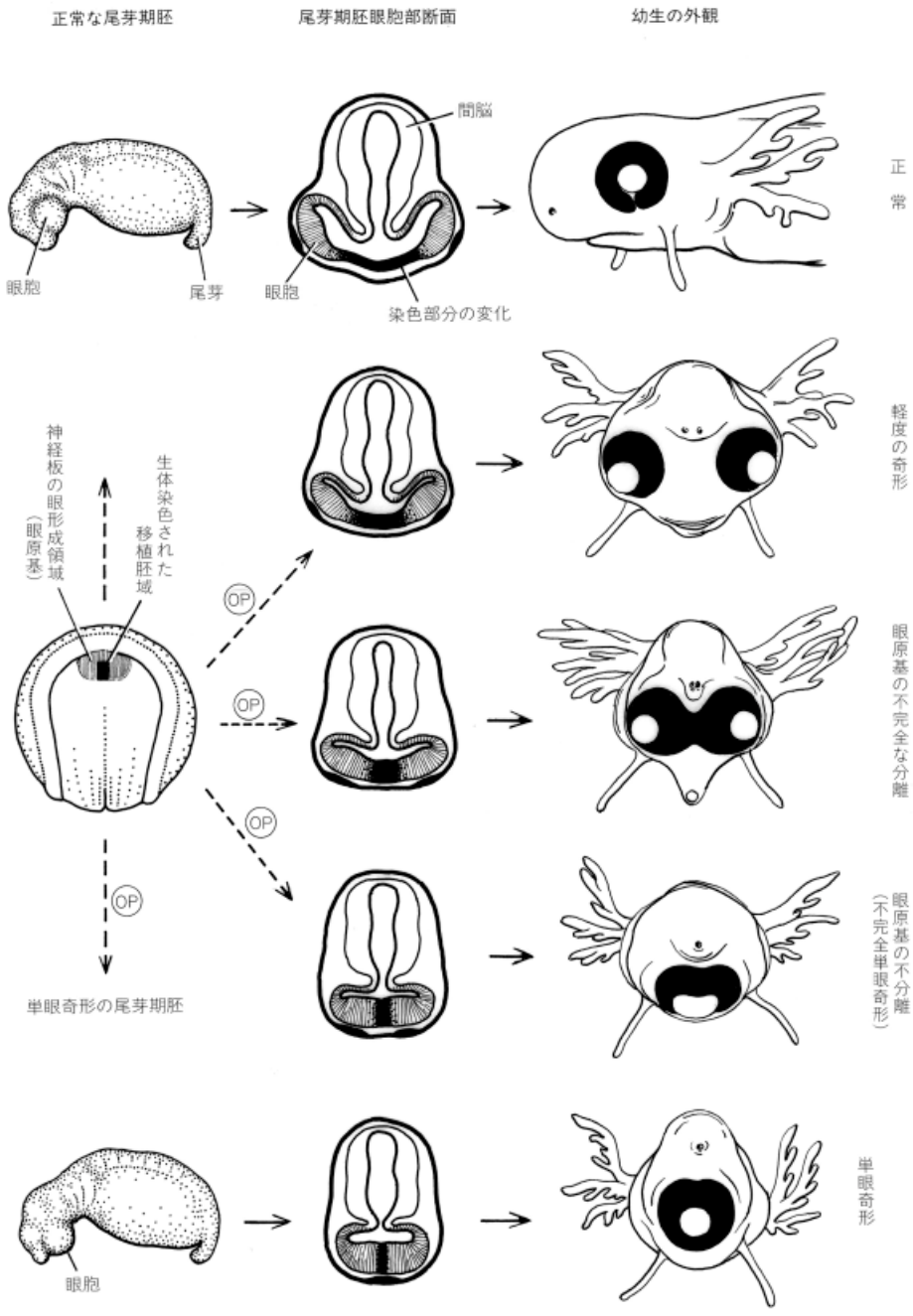

Fig. 4. Illustrations of cyclopean eye development. *This illustration forms part of a study by Goro Eguchi using a large number of larvae with cyclopeam eyes of different degrees of malformation.*

neural retina in adult newts resulted in a significant delay in the onset of the initial step of lens regeneration. These results could not be explained by Spemann's hypothesis, but they were encouraging enough for me to engage in the problem of lens regeneration.

You were a specialist in electron microscopy and had published several papers using this technique. What made you become a specialist in electron microscopy?

I was the first researcher to introduce electron microscopy to lens regeneration research. I thought that morphological changes of the iris after lens removal could be analyzed much more precisely by electron microscopy, because Wolff had described in his 1895 paper, more than a half century previously, that melanosomes discharged from dorsal iris epithelial cells might be engulfed by leucocytes. Professor Sato accepted my idea and decided to introduce a JEOL-T6 electron microscope to his laboratory in 1961. This was the first electron microscope in the Faculty of Science, Nagoya University. So, I began electron microscopic studies on lens regeneration at that time.

How did this new technique help your studies of lens regeneration? What did you find?

I analyzed successive morphological changes in iris epithelial cells (IEC) after lens removal and made important new findings. For example, macrophages from the circulatory system of the eye actively participate in dedifferentiation and depigmentation by their phagocytic activity. When an isolated piece of dorsal iris in a tiny diffusion chamber is cultured in the lentectomized eye, dedifferentiation and depigmentation of IECs is remarkably delayed with no involvement of the phagocytic cells, whose interaction with the IECs was completely blocked. By looking at more than fifty serial thin sections, I could see dramatic changes in mitochondria, as cells changed from dedifferentiated IECs to lens fiber cells, via IEC-derived lens epithelial cells. Globular mitochondria in both the dedifferentiated IECs and the IEC-derived lens epithelial cells fused with each other to form large and complex mitochondria, and they eventually elongated during lens fiber differentiation as they became distributed along the axis of differentiated lens fiber cells.

I understand that your electron microscopy led to a collaboration in another research field. Tell me what you found from that collaboration.

One day I met one of my colleagues, Dr. Sho Asakura, of the Institute for Molecular Biology at the faculty canteen and talked about our work. At that time Dr. Asakura was engaged in an approach to *in vitro* reconstitution of bacterial flagella in *Salmonella*.

It was already known that *Salmonella* flagella are organized as molecular assemblies of a single protein species, flagellin. Dr. Asakura had predicted that growth of *Salmonella* flagella must be a sort of crystallization. Through our discussions, he eventually proposed that I collaborate with him. I was very impressed and

interested in his idea, so I accepted his proposal; Professor Sato strongly encouraged our collaboration, which lasted from 1963 to 1968.

Based on his prediction, we designed the following experiment. Short fragments of flagella, which could be produced by ultrasonic treatment, were added to a supersaturated solution of flagellin molecules. If flagella could be reconstituted from fragmented flagella as seeds, in the same manner as crystal growth, we would be able to trace the process of reconstitution *in vitro* by electron microscopy, in which negatively stained specimens obtained successively from the reaction solution could be observed. We would also be able to conduct kinetic analysis of flagellar morphogenesis.

These experiments gave us the following results: 1) bacterial flagella can be completely reconstituted *in vitro* by adding fragmented flagella as seeds to supersaturated flagellin; 2) flagellin molecules synthesized in bacterial bodies are transported by free energy to the tips of the growing flagella, through the central tunnel of the flagella themselves, and polymerize at the tip; thus flagella grow unidirectionally; and 3) the length of flagella *in vivo* might be determined by a limited supply of flagellin molecules, which are thermodynamically transported from the bacterial body to the tip of the growing flagella.

What did you learn from this collaboration work?

My research ability improved tremendously through this collaboration. But also it contributed to my appointment as associate professor in the Department of Biophysics at Kyoto University, which I mentioned earlier. This collaborative experience allowed me, as well, to contribute significantly to the research of Professor M. Tokushige, Department of Chemistry, Kyoto University Faculty of Science, who had been studying enzyme-substrate reactions in *E. coli* aspartase between 1967 and 1977.

But, at that time, student protests were very active at Kyoto University. Were you able to do research?

Yes, when I was appointed associate professor in the Department of Biophysics, which opened in Kyoto University's Faculty of Science in 1968, and joined a research group led by Professor Okada together with my graduate student, Masatoshi Takeichi, unfortunately, student protests had spread to Kyoto University. I was appointed chairman of the committee for student problems, and so I had to consider how to conduct my experiments under such restricted conditions. I tried to apply a potent carcinogen, nitrosoguanidine (N-methyl-N'-nitro-N-guanidine), to the lens regeneration system of the newt to ask whether or not IECs can differentiate in tissues other than lens tissue.

I designed an experiment in which a microcrystal of the carcinogen, less than 10 micrograms, was administered into the eye cavity after lens removal. I thought that such a simple experiment could be conducted easily under such unusual conditions. I found that the nitrosoguanidine induced lens regeneration from the iris epithelium of the ventral iris, in addition to ordinal lens regeneration from the dorsal iris, without producing tumors or any kind of differentiation other than in lens tissue. This result also suggested that nitrosoguanidine, in this case, did not act as a mutagen or carcinogen, but instead modified cell surface activity by randomly binding cell surface proteins responsible for cell adhesion, movement, and so on.

You then started monolayer and clonal cell cultures to show transdifferentiation of pigmented epithelial cells into lens cells.

Based on the nitrosoguanidine result mentioned above, I immediately started cell culture analyses of pigmented epithelial cells from adult newts and from older chick embryos at 9 to 11 days of incubation. I could clearly show by clonal analysis that both dorsal and ventral iris epithelial cells from chick embryos invariably transdifferentiate into lens cells, to form lentoids expressing strong lens specificities *in vitro*. Professor Okada did not accept this result at first, but he eventually believed my well-controlled experiment, after careful inspection of my protocol and cell cultures, and proposed that we publish this cell culture study together.

Fig. 5. Goro Ecuchi, *President of Kumamoto University (2001).*

Culturing newt pigmented epithelial cells was much harder than culturing chick embryonic pigmented epithelial cells. Freshly dissociated adult newt pigmented epithelial cells did not begin to divide *in vivo* for more than 10 days. However, I eventually managed to get a clear result in the newt cells, with great patience and care.

By 1985 I had tested the lens transdifferentiation potential of pigmented epithelial cells of several kind of vertebrates, including reptiles, and came to conclusion that vertebrate pigmented epithelial cells must retain their ability to transdifferentiate into lens or neuronal cells as a dormant potential. By establishing a cell culture system using chick embryonic pigmented epithelial cells, my research group at NIBB (National Institute for Basic Biology)

extensively analyzed mechanisms of transdifferentiation and accumulated useful information for further studies, and I succeeded in lens and neuronal cell transdifferentiation of human pigmented epithelial cells from a 16-week fetus, and from 22- and 80-year-old adults.

Who among your students and collaborators are still working on lens regeneration?

In 1996, I was unexpectedly appointed President of Kumamoto University (Fig. 5), a national university founded in 1949, and I moved from Okazaki to Kumamoto in November 1996. This meant that I could not continue my research any more. However, many of my colleagues at Kyoto University, Nagoya University, the National Institute for Basic Biology, Okazaki, and other places, have continued to study problems of common interest. You yourself, Professor Yasuda, have pioneered molecular approaches to lens development in Japan. My former research associate and graduate students have pursued research into lens regeneration and transdifferentiation of PECs. For example, Professor P. A. Tsonis, University of Dayton, one of my graduate students at Nagoya University, has extensively developed lens regeneration research, and is now engaged in approaches to clinical application of a human PEC line, 80HdePE-Cb, which I established. He is now a leading contributor to research on regeneration in the United States.

Dr. Mitsuko Kosaka, my former research associate, collaborates with people in the Department of Ophthalmology, Kyoto University School of Medicine, studying neuronal cell transdifferentiation in 80HdePE-Cb cells, and she has also researched molecular and cellular mechanisms of transdifferentiation using the chick iris PEC experimental system, which she established herself. Dr. Makoto Mochii, another of my graduate students in Nagoya and Okazaki, and now an associate professor at Himeji Institute of Technology, has extensively developed his research on the role of the *Mitf* gene, responsible for transdifferentiation of PECs. In addition, Dr. Nobuhiko Mizuno, who was also one of my graduate students at NIBB, Okazaki, is now a research associate with Professor Hisato Kondoh, Osaka University. Professor Kondoh has paid special attention to the importance of the transdifferentiation phenomena, and recently started to study this problem as his new research project with Dr. Mizuno.

Are you still planning to remove lenses from the young newts which you have been culturing at your home for several years?

Any aspect of the lens regeneration problem is definitely still part of my life work. Even now I am culturing newts at home with the kind assistance of my wife, Yukiko. My most important questions are whether or not the newt can continuously regenerate lens tissue, and what are the key genes responsible for lens regeneration. Therefore, I am doing repeated lens removal experiments, and recently obtained the very interesting result that removing the lens more than 15 times results in a significant delay in the onset of the initial changes. I also succeeded finally in breeding newts which are as much as eight years old. Japanese newts take at least seven years to mature. Now, in my home, 8-year-old newt couples and their 8- to 10-month-old offspring are growing.

Thank you for giving me so much of your time for this interesting and fruitful interview. I'm looking forward to seeing your current work published with you as the first author in the near future!

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